ENGINEERING ANTIBODIES TO DETECT BIOLOGICAL WARFARE AGENTS

S. A. Goodchild *, N. A. E. Hopkins, T. E. Love, V. Patel, B. C Houghton and C. Mayers
Defence Science and Technology Laboratory (Dstl)
Dstl Porton Down, Salisbury, Wiltshire, SP4 0BY, UK

ABSTRACT

Antibodies play a critical role in detecting biological warfare agents, as they possess exquisite specificity and sensitivity to their targets. Antibodies for military use must possess additional qualities. They must be robust in order to survive field conditions and shipping, must integrate into fielded equipment and must be produced quickly and cheaply. Recent advances in recombinant antibody technology provide the opportunity to produce cheaper detection antibodies, specifically engineered to improve their utility in a wide variety of detection platforms.

1. INTRODUCTION

The technologies used for detecting biological warfare agents are diverse and rapidly changing, with many new sensor technologies currently in development. Biosensors are required that provide real-time detection of biological warfare agents, and are likely to gain their specificity and sensitivity from antibodies incorporated into the biosensor.

Immunoassays are a popular way to provide fast, accurate identification of agents, including assays such as enzyme linked immunosorbent assay (ELISA), lateral flow hand held test tickets, electrochemiluminescence and surface plasmon-based methods. All immunoassays rely on antibodies to provide specific, fast detection of their target. As a result any improvements that can be made to these antibodies leads directly to improvements in the assays in which they are used

1.1 Polyclonal antibodies

The easiest way to produce antibodies for immunoassays requires immunisation of animals with a target protein or killed agent. After several months of repeated immunisations, serum containing antibodies is collected from the immunised animals. The animal used is dictated by the size of the batch of assays being produced; mice are frequently used for one-off laboratory scale assays, while rabbits, sheep and goats are used for larger batch sizes. The work is expensive, as it covers the maintenance of these animals through the immunisation process.

An antibody prepared from these immunised animals is referred to as a polyclonal antibody. A polyclonal antibody contains all of the antibodies that the animal was producing at the time the blood sample was taken. Polyclonal antibodies are often used in assays to provide an extremely high sensitivity. The complex mixture of antibodies can however also cause difficulties. As well as containing all the desirable antibodies produced in response to the immunisations, the serum also contains antibodies to other substances that the animal may have been exposed to during its lifetime. As a result a polyclonal antibody may produce a high occurrence of false positive results to unrelated targets. In addition, batch-to-batch variation occurs in polyclonal antibody production, as not all animals will respond to the target in the same way. The specificity of polyclonal antibodies can be improved by purifying the antibodies that are specific to the target by affinity chromatography (Petrenko and Volyanoy, 2003). This approach is not however applicable to all antibodies.

1.2 Monoclonal antibodies

Polyclonal antibodies contain of a mixture of antibodies produced by a multitude of immune cell clones in the animal's immune system. It is possible to isolate single cell clones from an animal and immortalise them by artificially fusing them with an immortal cell line. These immortalised antibody producing cell lines (hybridomas) can be grown in the laboratory by cell culture, and continue to secrete antibodies to their growth medium. The antibodies are produced by a single clone of identical cells, so are referred to as monoclonal antibodies. Monoclonal antibodies present an easily characterised, highly pure antibody, although their creation is often slow and relatively expensive. The cell lines produced must be stored carefully, production of antibody can be variable, and problems can occur with cell lines mutating or ceasing to secrete antibodies.

1.3 Recombinant antibodies

Although an antibody is a relatively large protein, only a small region is required to bind its target. Each antibody has two of these antigen-binding regions, each of which is around 1/5 of the size of a whole antibody.

Antibody genes encoding these binding regions can be isolated from immunised or naive animals and, after

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
00 DEC 2004	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Engineering Antibodies To Detect Biological Warfare Agents	**g**
	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Defence Science and Technology Laboratory (Dstl) Dstl Porton Down, Salisbury, Wiltshire, SP4 0BY, UK**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
See also ADM001736, Proceedings for the Army Science Conference (24th) Held on 29 November - 2 December 2005 in Orlando, Florida., The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**6**	

some modification, be introduced into a bacterial expression system. These bacterially produced antibodies are referred to as recombinant antibodies, and can be produced rapidly in large quantities by a simple fermentation process. The cell lines used (typically *E. coli*, although other bacteria and yeasts have been used successfully) are cheap and easy to grow, reducing the cost of antibody production significantly.

Probably the biggest advantage of recombinant antibodies is that once isolated, the antibody genes can be modified to change the properties of the antibody. This is often used to improve the binding affinities of the antibodies, giving an enhancement in assay sensitivity, but can also be used to facilitate their attachment to biosensor surfaces or to add reporter enzymes. The properties of the antibodies can be altered significantly by this process, to improve their level of expression, multimerisation, or size.

We have produced recombinant antibodies to biological warfare agents and simulants using an M13 phage-display system. By using large libraries, specific antibodies can be selected within a few weeks. By modifying the methods of biopanning used we could select for highly specific antibodies that do not react with closely related, non-infectious species of bacteria. We have used the same system to produce antibodies to smaller ligands and haptens. It is possible to improve these antibodies by random and directed mutagenesis to improve their utility in biosensors, and we have successfully used recombinant antibodies to give real time detection on biosensor surfaces.

2. PRODUCTION OF AGENT-SPECIFIC ANTIBODIES

The starting point for producing recombinant antibodies is to produce a library of antibody genes in a host organism, usually *E. coli*. These libraries can be produced artificially by gene synthesis, or can be produced from a single immunised mouse. In the phage-display system, the library is infected with M13 bacteriophage, producing recombinant bacteriophage with the antibody displayed on its surface (Krebber *et al.*, 1997). Once the library has been produced it can be screened (panned) against a chosen antigen. The selection is usually carried out for several rounds of panning to enrich for specific antibodies (Figure 1). The panning process can be improved further by pre-absorbing the bacteriophage library with antigens to remove any potentially cross reactive antibodies present (competitive panning). Competitive panning (Krebs *et al.* 2004) is an extremely powerful and fast technique - in a few days we used it to isolate recombinant antibodies to anthrax spores that do not cross react with any other *Bacillus* species. We have successfully used this panning strategy to isolate antibodies to a wide range of agents, from intact viral and bacterial threat agents to toxins, small haptens and chemicals.

3. USING RECOMBINANT ANTIBODIES IN DETECTION TECHNOLOGIES

Recombinant antibodies can be used successfully in any technology where conventional antibodies are used, with the added benefit of reduced production cost and the ability to modify and improve antibodies to suit the technology. We have successfully used recombinant antibodies in immunoassays such as ELISA (Figure 2), hand held lateral flow assay (Figure 3) and on surface plasmon based biosensors such as the Biacore biosensor (Figure 4).

4. MODIFYING RECOMBINANT ANTIBODIES TO IMPROVE UTILITY IN SENSORS

Once a specific antibody has been found it can be modified to improve its utility in the detection technology. We are developing specific tags and fusion proteins designed for each detection technology, for example, tags designed for successful immobilisation on biosensor surfaces, or for immobilisation on gold sol for lateral flow assays. This ability to modify antibodies also allows the physical properties of the antibodies to be altered. We have developed fusion proteins that greatly reduce the propensity of the antibody to precipitate in 'difficult' buffers, and have improved the stability of antibodies in organic solvents (Figure 5). It is also possible to improve the affinities of recombinant antibodies by random or directed mutagenesis to increase the sensitivity of assays (Figure 4).

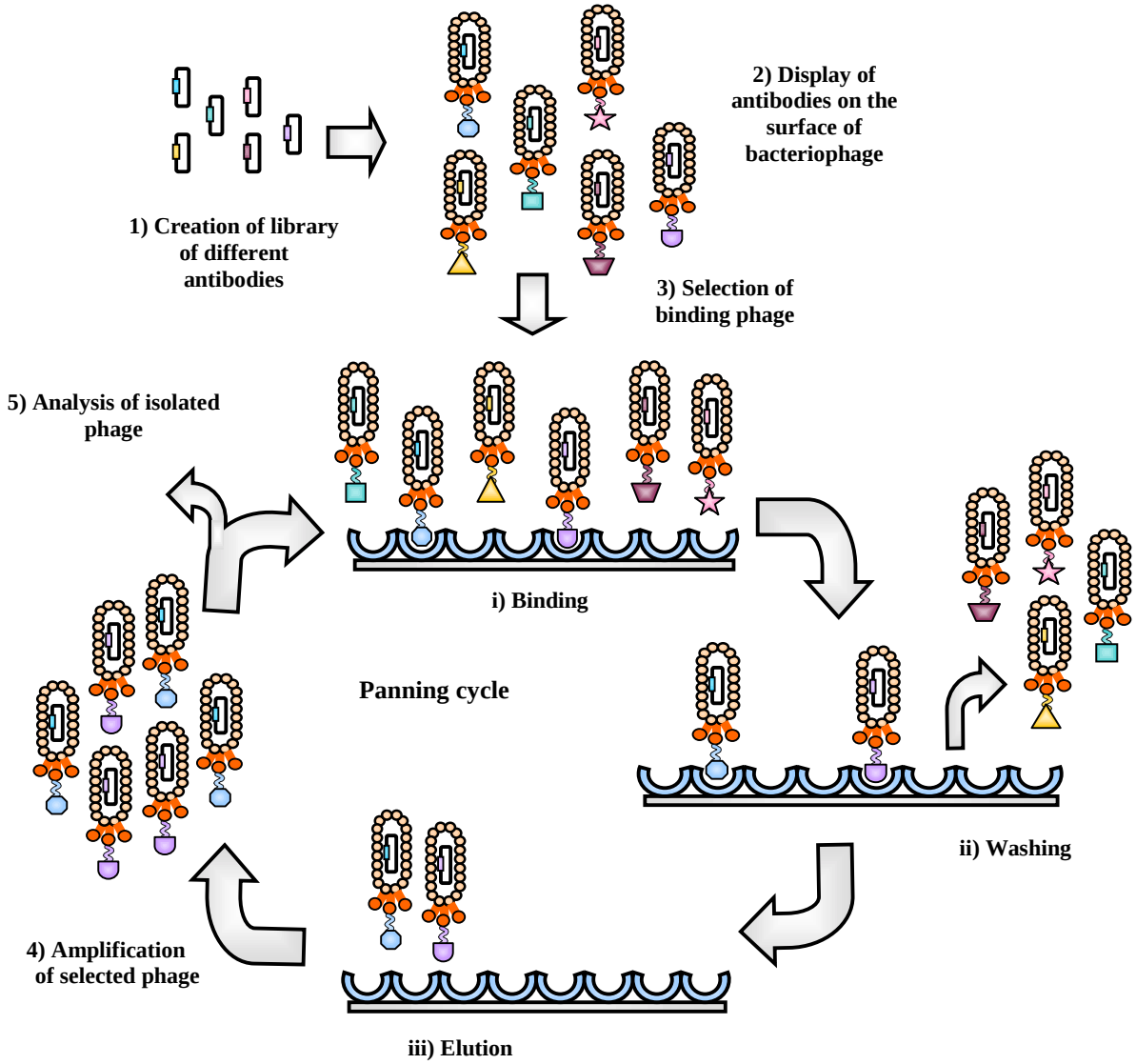

Figure 1. Schematic representation of the steps involved in panning a phage-displayed antibody library

1) DNA encoding millions of different antibodies is batch cloned into the genome of filamentous phage linked to one of the phage coat proteins. **2)** Each DNA variant is packaged into a separate phage particle. The cloned fragment is displayed on the phage coat protein upon expression of the fusion construct. **3)** Selection of these phage using an immobilised target molecule (bacterium, virus, protein or ligand) allows enrichment of phage which bind to the desired antigen through cycles of **i)** binding, **ii)** washing and **iii)** elution. Phage with no binding affinity towards the target molecule will be washed away leaving those that display a ligand of interest. Bound phage can be recovered by non-specific elution such as pH change or competitive elution using a competitive binder to the ligand of interest. **4)** The phage of interest can be reinfected into bacteria and the new library amplified for further rounds of selection. **5)** Clones from the enriched library can be analysed for binding properties using ELISA, SPR or other techniques.

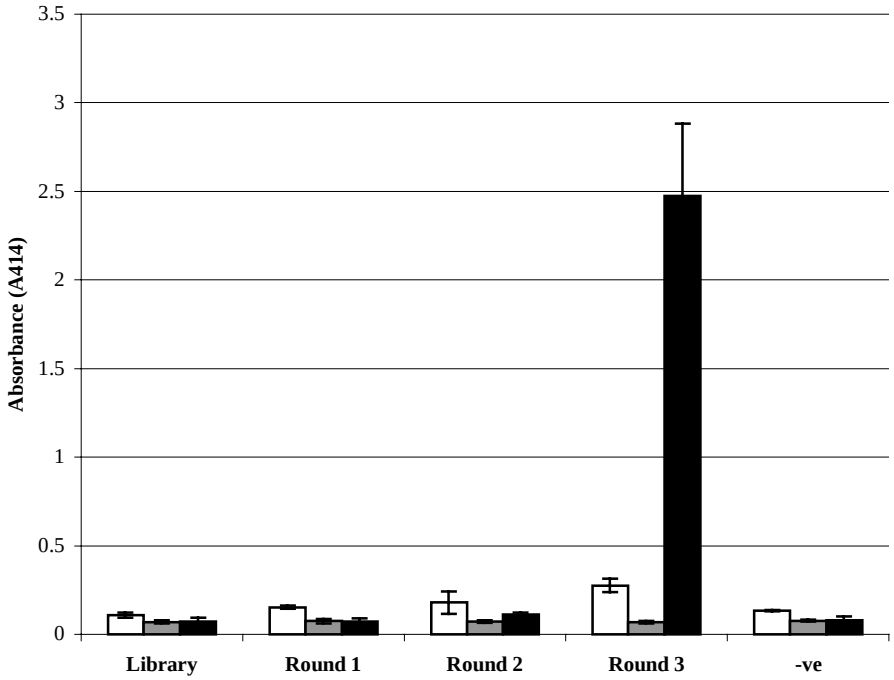

Figure 2: ELISA results testing recombinant antibodies against bacterial spores.

Recombinant antibodies were produced both from an immune mouse recombinant antibody library (Library), and from rounds of biopanning against bacterial spores (Round 1, 2, 3). A recombinant antibody to ovalbumin was used as a negative control (-ve). The response against the target bacterial species is shown in black, the response against a related species is shown in white, and the response against a negative control antigen (dry milk powder) is shown in grey. Each panning round selects for antibodies specific to the target, with little or no selection for the control antigens. Error bars represent the standard deviation of three samples.

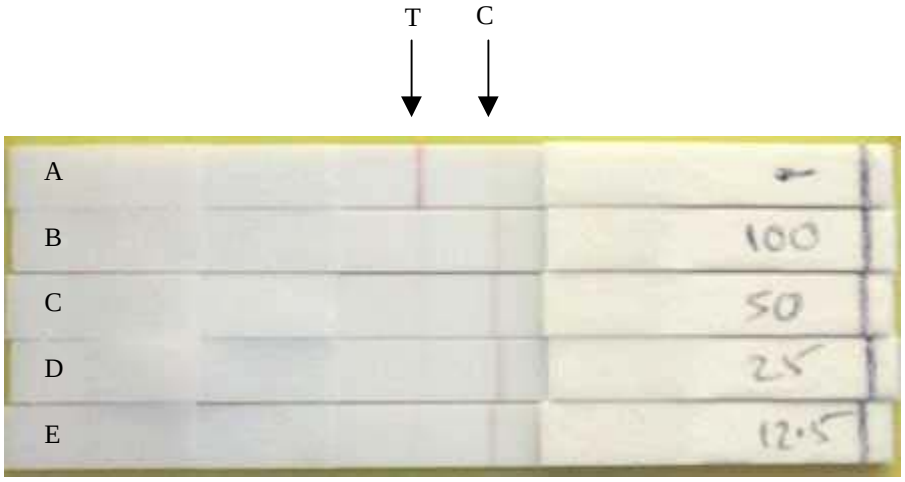

Figure 3: Recombinant antibodies used in a competitive lateral flow immunoassay

Recombinant antibodies against a hapten were used to coat gold for use in competitive lateral flow immunoassay. The test line (T) is coated with hapten, while the control line (C) is coated with an anti-His tag antibody. In the absence of hapten (strip A) the antibody-gold complex sticks to the hapten strip, indicating a negative result. In the presence of hapten (strips B-E) the free hapten binds to the gold sol, and prevents it from binding the test strip, instead being captured by anti-His

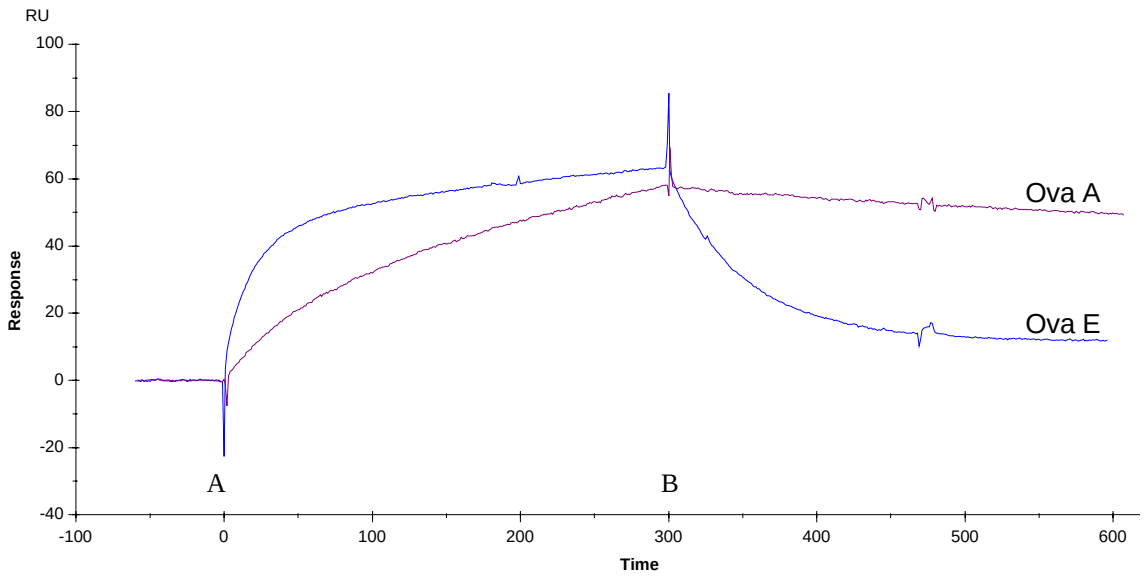

Figure 4. Recombinant antibodies giving real time detection on a biosensor

Antibodies to a toxin simulant were immobilised on the surface of a Biacore sensor chip and surface plasmon resonance measured as the chip was exposed to the toxin simulant. Chips were exposed to buffer before t = 0, and exposed to the simulant at t = 0 (point A). An immediate response occurs on exposure to the simulant; the x axis scale is in seconds. Two different antibodies have been used - OvaA has a higher affinity, but a relatively slow on rate. OvaE has a lower affinity, but has a much faster binding rate, allowing greater sensitivity. OvaE also releases the simulant after exposure to the simulant stops (point B), allowing easy regeneration of the sensor. Mutagenesis of recombinant antibodies allows the manipulation of binding rates and affinities to suit different sensor applications.

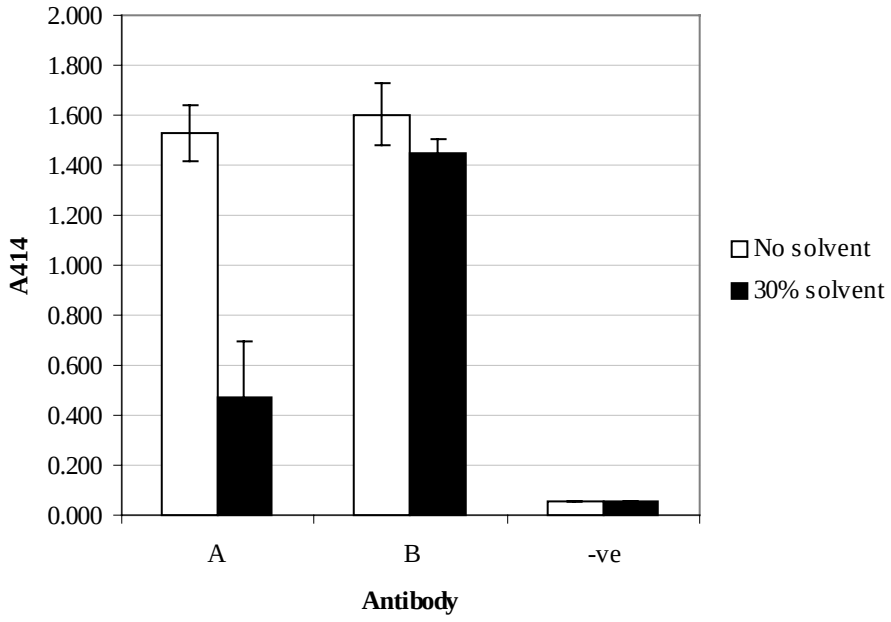

Figure 5. ELISA showing in-vitro evolution of antibodies selected to be stable in organic solvent

Recombinant scFv A is able to recognise a hapten in aqueous solution, but has poor binding when organic solvent is present. ScFv B was mutagenised to create 10^7 variants, and panned in a solvent mixture to select stable variants (scFv B as an example). This result shows that it is possible to greatly increase stability in hostile environments by in-vitro evolution.

CONCLUSION

We have found that recombinant antibodies present significant advantages over conventional antibodies in detection technologies. Their selection is quicker, they reduce or eliminate animal use, and cost less to develop. They are cheaper to produce in large quantities, can be produced on a 'just in time' basis due to their speed of production, and can be modified to improve their characteristics in different sensors. The new generation of biosensors will demand high performance, high affinity antibodies that can be accurately characterised and modified. We believe that recombinant antibodies will fulfil this demanding role.

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